11

Exposure and Biomarker Assessment in Humans

The evaluation of potential reduced-exposure agents (PREPs) has to be defined in the context of the outcome of interest (e.g., individual or population reduction in risk and disease type) and compared to an appropriate baseline (i.e., nonsmokers, former smokers, current smokers in the context of host susceptibility and previous level of smoke exposure). Tobacco exposure can be measured in the aggregate at the level of the entire population (e.g., through the measurement of tobacco sales or reported consumption in population-based surveys) and related to disease incidence or change in mortality rates. These methodologies are considered descriptive epidemiological tools that are useful in generating hypotheses and/or validating public health strategies, marketing programs, and so forth. Exposure can also be measured at the level of the individual through biomarker measurements. This type of assessment within epidemiological studies can be used for hypothesis generation or testing. A range of methodologies and assays can be used for assessing exposure, as well as a range of assays for assessing host susceptibilities to exposure.

The evaluation of a PREP can include four components: (1) external exposure measurements, (2) internal exposure measurements, (3) biomarkers estimating the biologically effective dose (Perera, 1987), and (4) biomarkers of potential harm (see Figure 11-1). The definitions of each are provided in Table 11-1 and explained further herein. There have been different definitions of types of exposure assessments used previously, but more recent understandings of biomarker uses and limitations, as well as different approaches needed for PREP evaluation lead to a need for clarification and redefinition. The latter three measurements improve upon the first by quantifying exposure at the cellular level to characterize low-dose exposures or low-risk populations, providing a relative contribution of individual chemical carcinogens from complex mixtures and estimating total burden of a particular exposure where there are many sources (Vineis and Porta, 1996). In assessing PREPs through biomarkers, understanding the biological effects of a wide range of exposures will be important. Within the context of this chapter, exposure at the level of the cell and critical macromolecules is considered with greater weight, rather than the traditional view of exposure at the portal of entry into a person.

Biomarkers are intuitively more informative and better disease risk markers when measured in the target tissue through biopsies (e.g., oral mucosa , lung , bladder). However, biomarker assays are technically limited, and target tissue can be difficult to obtain, especially in nondiseased smokers. Therefore, biomarker assays have been developed for surrogate tissues and fluids (e.g., expired breath, saliva, blood, urine). While these are technically simpler to use and easier to collect, the ability to prove a predictive value for the potential harm reduction is more difficult. It should be noted that the biomarkers discussed in this chapter refer to either target or surrogate tissue or fluid assays, but that the biologically effective dose refers to the assessment of a mechanistically relevant biomarker only in the target organ.

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The following factors should be considered when evaluating measurements for predicting or determining the effects of a PREP. Table 11-2 summarizes these factors and Table 11-3 provides an overview of available measures to predict the effects.

1. *Type of measurement.* Measurements are defined within four general categories—namely, external exposure, biomarkers of exposure, biomarkers estimating the biologically effective dose, and biomarkers of potential harm. Placing a measurement solely within one category may not be possible. The external exposure assessment category is limited to those methods that are not detected by an assay using a body fluid or part. Although some external exposure methods might be poor predictors of disease risk and hence also poor measures for assessing a new product (e.g., the Federal Trade Commission [FTC] method described below), others might be strongly associated with disease risk and might therefore be better (e.g., cigarettes per day). While some external exposure assessments might be useful for harm reduction risk assessments (e.g., smoking history), they should not be used alone in assessing harm reduction because the predictive power for disease is not sufficient without corroborative biomarker data. Biomarkers of exposure are assayed in a body fluid (including exhaled air) or tissue that measures a constituent of tobacco smoke, tobacco-related products, or metabolites, where the constituent is not bound to a biomolecule. These biomarkers include unmetabolized compounds (e.g., carbon monoxide [CO], serum nicotine, carcinogen levels in serum or internal organs), biomarkers of exposure to individual cigarettes (e.g., incremental increases in exhaled CO or serum nicotine), and metabolites in any body fluid (e.g., cotinine in serum or urine, carcinogen metabolites in urine). Biomarkers assessing the biologically effective dose are those considered mechanistically related to disease outcomes (e.g., carcinogen–DNA adducts in the target tissue). Surrogate biologically effective doses, once validated, estimate a biological effect in a target organ (e.g., hemoglobin adducts or white blood cell carcinogen–DNA adducts). These biomarkers are in theory best able to link exposure (external and internal) to disease outcomes. Biomarkers of potential harm can reflect early or late damage (e.g., loss of heterozygosity in sputum, background mutations in nondiseased tissues, reactive airway disease, arrhythmias, premalignant lesions, mutations in premalignant lesions, chromosomal aberrations in smoking-damaged epithelium, hypermethylation of genes, atherosclerosis). In this context, potential harm implies that the assay might or might not reflect actual harm and that some change in physiological function, for example, might not represent a harmful effect.

2. *Target tissue and outcome relationship.* A biomarker assay should be shown to be relevant to the outcome of interest. Besides having a mechanistic relationship to pathogenesis, data should be available to determine the predictive capacity for disease and disease reduction. This validation includes supportive evidence that the assay reflects harm reduction, such as might be done in an experimental cell culture or animal study. Assays that measure the effects in target tissue would generally have the greatest weight to support the use of a PREP. Sometimes, the target tissue effect might also be a surrogate for an effect in other tissues, and a surrogate tissue assay might reflect effects in multiple organs.

3. *Dose–response data for harm.* Assays that have a demonstrable dose–response relationship to actual disease outcomes is important for assessing a PREP and, if they do not, it should be shown to have a dose–response relationship to a biomarker of potential harm relevant to a disease pathway. The mechanistic basis for the relationship should be well understood in order to make meaningful interpretations of data used to assess a PREP. For example, assays that demonstrate a dose–response relationship between smoking and DNA damage in epithelial cells of a

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target organ could be useful. Methods assessing tobacco exposure as a complex mixture would have greater weight than a single component exposure.

4. *Dose–response data for harm reduction.* Assays that show a reduction in harm after reducing exposure to tobacco smoke or a tobacco product constituent would have the greatest weight, where the experimental design uses an initial dose level for a specific duration of time followed by exposure to a lower level at a later time. The intent is to simulate the effects of a person's switching from one level of exposure to another level of exposure. Importantly, the effects of the biomarker should be measurably different over the range of human exposures, so that the assessment can predictably measure the effects of exposure reduction from a PREP. Currently, there are some biomarker assays that have been assessed in former smokers or smoking cessation trials. These biomarker studies that indicate measurable decreases in effect can provide some information about the utility of markers for assessing exposure reduction. Included in this are half-life data, which must be measured and taken into account when evaluating a tobacco-related PREP. Methods assessing tobacco exposure as a complex mixture would have greater weight than a single component exposure.

5. *Specificity.* Consideration should be given to whether the effect is specific to a constituent of tobacco smoke or a tobacco product, or whether the method also measures exposure from other sources. Higher degrees of specificity are useful, although in some cases the method might be useful for assessing exposures from multiple sources other than tobacco in order to provide an understanding about relative contributions. Assays that are specific for tobacco's complex chemical mixture and those that are specific for a chemical or chemical class both have utility, but the former would have greater weight if appropriately validated, because persons are exposed simultaneously to all of the constituents. Validating assays for complex effects is more difficult because they may have less specificity for tobacco.

6. *Sensitivity.* The assay must be sufficiently sensitive to measure what it is supposed to measure in the human tissue of interest. This is especially problematic in measuring low-level effects, for example, in assessing the effects of environmental tobacco smoke (ETS) exposure.

7. *Validation.* It is critical that biomarkers for assessing PREPs be well validated in the laboratory. Validation includes proof that the assay measures what it claims to measure and that it is reproducible. Sensitivity, specificity, and predictive value are all important to consider.

EXTERNAL EXPOSURE ASSESSMENT: THE FTC METHOD AND QUESTIONNAIRE DATA

External exposure markers attempt to measure the amount of a tobacco smoke or tobacco product constituent that may enter at a portal to the body. However, these predictors generally do so without regard to most interindividual differences in smoking behavior and cellular processes. There are several types of external exposure assessment, some of which are listed in Table 11-4.

A common way to assess potential exposure to tobacco smoke is by measuring the yield of tobacco smoke constituents. One attempt to estimate delivered doses is the method adopted by the Federal Trade Commission in 1967. It was intended to provide a standardized estimate of tar and nicotine yield by cigarette brand, simulating a cursory observation of human smoking behavior. A cigarette is inserted into a smoking machine and lit, puffs are taken through a syringe (35 ml over 2 seconds, every 60 seconds) until the cigarette is "smoked" to a fixed length. Particulates are collected on a filter and weighed. Nicotine is assayed separately. Tar is measured as total particulate matter less nicotine, other alkaloids, and water. Although the machine provides yield data that can be used to compare one cigarette to another, this information has limited use-

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fulness for understanding human exposure because people do not smoke cigarettes as the machine does due to different smoking behaviors. Smokers also can affect cigarette filter performance by covering ventilation holes in the filter with their lips or fingers, which would increase yields in vivo. Although FTC yields might define a comparative range of actual exposures, there is a wide overlap of actual to predicted yields among types of cigarettes (i.e., low, medium, and high yields), where smokers of low-nicotine cigarettes might have higher nicotine levels than those who smoke brands with higher FTC yields (Byrd et al., 1998, 1995). Altering the FTC method to simulate puffs and times for actual smokers results in higher exposures to tar and specific carcinogens (e.g., tobacco-specific nitrosamines and benzo[a]pyrene) (Hoffman and Hoffman, 1997; Fischer et al., 1989; Djordjevic et al., 2000). For example, using modified protocols to stimulate human smoking behavior, the medium-yield (0.9–1.2 mg nicotine per cigarette) and low-yield (0.8 mg nicotine per cigarette) cigarettes deliver similar amounts of tar per day, although by FTC method measured per cigarette yields of tar, benzo[a]pyrene [BaP], and tobacco-specific nitrosamines (TSNAs) were higher in the former (Djordjevic et al., 2000). As cigarettes with different designs are developed and marketed, an assumption that the FTC method of estimating yields will be comparable to existing products is premature.

Over the last 30 years, data from surveys have been an important tool in the assessment of tobacco exposure among individuals and the population. They have been an effective means of tracking patterns of tobacco use and the societal perceptions that ultimately influence consumption. Individual exposure can be assessed through the measurement of the number of cigarettes smoked per day, duration of smoking, types or brands of cigarettes smoked (e.g., "tar" delivery, filter type, type of tobacco, mentholation), and age at initiation (U.S. DHHS, 1988; International Agency on the Research of Cancer, 1986; Zang and Wynder, 1992; La Vecchia et al., 1990; Kaufman et al., 1989; Stellman and Garfinkel, 1989; Wilcox et al., 1988; Lubin et al., 1984 Vutuc, and Kunze, 1983). Lifetime exposures can be estimated by calculating pack-years (average packs per day multiplied by number of years smoked) or cumulative tar exposure (Zang and Wynder, 1992). A more detailed description of the most common surveys in use is presented in Table 11-5.

Most analyses indicate that self-report validity among adults is good (Patrick et al., 1994). Certain limitations, however, are evident in this type of exposure assessment (U.S.DHHS, 1994; Giovino, 1999). First, sampling errors may occur in any study in which generalizations are made from a selected population sample. One example is the over- or underrepresentation of certain groups, especially those that exhibit significant tobacco use or have differing smoking behavior. In fact, there is a built-in exclusion in many of the major surveillance tools of various segments of the population, such as the institutionalized mentally ill, prisoners, and those in areas of inadequate telephone coverage. Errors in response must be considered including memory errors, nonresponse errors, and misclassifications and inconsistencies in reporting. The validity of self-reported responses can be influenced by many factors (Velicer et al., 1992), particularly the respondent's perception of privacy (Giovino, 1999). This is especially a concern among adolescents in the home setting and among groups that have increased pressure to abstain or to quit, including pregnant women, adolescents, and patients with heart or lung disease. One effort to validate self-report measures and to reveal any ETS exposure can be found in the National Health and Nutrition Examination Survey (NHANES; see Table 11-5), which collects serum cotinine levels of respondents (SAMHSA, 1998; CDC, 1999; Giovino, 1999; Giovino et al., 1995).

Population surveys have limited practicality in evaluating the consequence of tobacco exposure because of the relatively long time frame required. However, in context, population assess-

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ments have been studied extensively in relation to disease outcomes and thus can be considered a crude measurement of individual risk and a better measure of population risk. These surveys do provide insight into trends of tobacco product use within and across a variety of sociodemographic groups, including age, sex, race or ethnicity, educational status, and economic status. The data can be compared to morbidity and mortality registries to understand new or changing consequences of use patterns or specific products. In addition, these trends in prevalence, initiation, and cessation in turn aid in the evaluation of the effects of tobacco-related activities, policies, and interventions within the general population and its subgroups.

Methods for assessing external exposure (e.g. number of cigarettes per day) are widely used and relatively inexpensive but do not provide an assessment of how someone smokes cigarettes and how the body responds to exposure. Thus, these measures approximate the level of actual exposure and, as described below, become less reliable in assessing exposure reduction. Smoking topography is an additional method of assessing external exposure (e.g., how much smoke enters the lung, estimated by measuring puff volume, number of puffs per cigarette, puff duration, total inhalation time, and interpuff interval) (Bridges et al., 1996; Herning et al., 1993; Gritz et al., 1983; Kolonen et al., 1992; Hofer et al., 1992). In the laboratory, if subjects smoke their own cigarettes, then it is presumed that the measurement reflects their usual smoking behavior. A limitation of smoking topography studies is that cigarettes are typically smoked via cigarette holders, which may influence puffing behaviors and prevent vent hole blocking that might normally occur when the cigarettes are smoked without the holder. Smoking topography studies have contributed to the findings that persons who switch from high-tar and nicotine to low-tar and nicotine cigarettes increase their intake of smoke per cigarette to compensate for a lower yield of nicotine (Benowitz et al., 1986a,b). It is well established that smokers self-titrate their blood nicotine levels, such that smokers of lower-nicotine cigarettes inhale more, (Gritz et al., 1983; Benowitz et al., 1986b; Benowitz et al., 1998; Hill and Marquardt, 1980; Ebert et al., 1983; Benowitz et al., 1983) and altering topography leads to differences in nicotine absorption and CO boosts (Kolonen et al., 1992a; Hofer et al., 1992). Smoking lower-nicotine delivery cigarettes increases puff volume (Bridges et. al., 1986; Kolonen et al., 1992b; Battig et al., 1982) and. to a lesser extent, puff duration (Bridges et al., 1990). Using a multiple regression model for prediction of nicotine blood levels, the best-fit model incorporates interpuff interval, number of puffs per cigarette, puff volume, puff duration, inhaled volume, and inhalation duration (Herning et al, 1983). These studies are difficult to interpret, however, because cigarette and topographic parameters are interrelated (Kolonen et al., 1992a; Bridges et al., 1990; Nemeth-Coslett and Griffiths, 1984).

Different methods have been developed for the study of exposure to environmental tobacco smoke (EPA, 1993). Stationary and personal air monitors can be used to measure total particulates or individual constituents. Some measurements, such as nicotine, are more specific for ETS. Ambient air concentrations and personal exposures to polycyclic aromatic hydrocarbons (PAHs) and other tobacco constituents can be to measured, but their relationship to disease risk has not been adequately studied.

BIOMARKERS OF EXPOSURE

Biomarkers of exposure, measured in a body fluid, tissue, or exhaled air, represent an internal dose of tobacco smoke or a tobacco product constituent that is either the parent compound or its metabolite. They are not measurements of how the constituents interact with body functions or macromolecules to cause harm. Some of these markers have been researched extensively, and

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they are more representative of actual human exposures to tobacco products than external measures of exposure. They are generally technically feasible and provide information about short-term (e.g., from a single cigarette) and long-term exposures. Examples are listed in Table 11-6, which gives a range of assays available but is not intended to be all inclusive. Because it has such a short half-life, carbon monoxide is best used for assessing recent exposures, although CO measurements also have been used to improve long-term exposure estimates of cigarette consumption (Law, et al., 1997). The limitations of CO are that there are other sources of carbon monoxide, such as automobile exhaust and endogenous metabolism, and there is some variation with differences in physical activity, gender, and the presence of lung disease or other disease states. Nicotine blood levels are used and are helpful for assessing internal exposure primarily because it has a very short half-life. Serum, urinary, or salivary cotinine, which is a metabolite of nicotine with a longer half-life, however, has been extensively studied for confirmation of exposure in smokers, quitters, and persons exposed to ETS (Bono et al., 1996; Benowitz, 1999; Crawford et al., 1994). Cotinine levels are dependent on both the extent of formation from nicotine by cytochrome P450 (CYP) 2A6 and the rates of oxidation and glucuronidation of cotinine to 3-hydroxy-cotinine and glucuronide conjugates, respectively, which vary widely among individuals. Therefore, cotinine levels are only approximately correlated with the daily intake of nicotine. Carbon monoxide and nicotine boosts (i.e., the difference between levels before and after a single cigarette), reflect smoking topography and exposures from an individual cigarette.

Technologies exist for directly measuring internal exposure to tobacco smoke constituents in target organs through biopsies (e.g., PAHs in the lung) (Lodovici et al., 1998) and for measuring levels of metabolites of compounds (e.g., those from TSNAs in the urine) (Carmella et al., 1995, 1993; Atawodi et al., 1998). Tobacco smokers have higher levels of mutagens circulating in the body, which can be measured by using extracts of urine in the Ames *Salmonella* mutation assay (Yamasaki and Ames, 1977; Jaffe et al., 1983; Mohtashamipur et al., 1985). Levels have been found to decrease with some test cigarettes that heat, rather than burn, tobacco (Smith et al., 1996).

The assessment of smoking exposure using nicotine or cotinine cannot be done in smokers who are concomitantly using nicotine replacement products. An alternative is to determine the levels of other tobacco alkaloids, such as anatabine or anabasine in the urine (Jacob et al., 1999).

BIOMARKERS ESTIMATING THE BIOLOGICALLY EFFECTIVE DOSE

The biologically effective dose (Perera, 1987) is the amount of a tobacco smoke or tobacco toxin that measurably binds to, or alters, a macromolecule (e.g., protein or DNA) in a cell. In some cases, the macromolecule may be a surrogate for a target molecule. The biologically effective dose represents the net effect of metabolic activation, decreased rate of detoxification, decreased repair capacity, loss of cell-cycle checkpoint control, and decreased rates of cell death. It should be noted that not all binding to, or alteration of, a macromolecule leads to an adverse health effect; so, often, what is really measured is the dose to a target macromolecule that estimates the biologically effective dose. Table 11-7 provides examples of biomarkers that estimate the biologically effective dose, but is not intended to be all inclusive.

Many tobacco-related toxins and chemical carcinogens are biologically inactive until transformed by cellular enzymes such as cytochrome-P450s into reactive intermediates. These reactive intermediates bind to macromolecules such as DNA and protein and disrupt their normal processes.

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For cancer, a common assessment of the biologically effective dose is the measurement of carcinogen–DNA adduct levels. These are formed when carcinogen metabolites are alkylated to nucleotides, creating a promutagenic lesion. There are strong laboratory animal data and some human studies that indicate a relationship between tobacco smoke constituents, carcinogen–DNA adduct formation, and cancer (La and Swenberg, 1996). Laboratory animal studies have shown a correlation between cancer and increased adducts in target organs (Nakayama et al., 1984; Ashurst et. al., 1983; Pelkonen et al., 1980). In humans, tobacco smoking leads to increased adduct formation in target tissues such as the lung (Schoket et al., 1998; Wiencke et al., 1995; Phillips et al., 1988) and in surrogate tissues such as blood (Wiencke et al., 1995; Vineis et al., 1994; Tang et al., 1995). Evidence exists that carcinogen–DNA adduct levels in target and nontarget organs are modulated by interindividual differences (Kato et al., 1995; Ryberg et al., 1997; Rojas et al., 1998; Badawi et al., 1995; Stern et al., 1993; Grinberg-Funes et al., 1994; Pastorelli et al., 1998). Interestingly, in former smokers, age of initiation may influence lung adduct levels (Wiencke et al., 1999). In humans, only a few studies have investigated a link between carcinogen–DNA adducts and cancer risk. All data come from case-control studies of the lung and bladder, and almost all show a positive relationship (Tang et al., 1995; Dunn et al., 1991; van Schooten et al., 1990; Peluso et al., 1998). However, since no published prospective studies of tobacco smoking show a relationship of adducts to cancer, the case-control studies must be interpreted cautiously because there may be an effect due to differential metabolism or DNA repair. The utility of carcinogen–DNA adduct measurements in assessing harm reduction is suggested by studies showing that lung adduct levels are lower in persons who smoked filter cigarettes (van Schooten et al., 1990). Hemoglobin adducts, an estimate of the biologically effective dose, are higher in smokers than in nonsmokers (Bryant et al., 1987), and in those who smoke black rather than blond tobacco (Bryant et al, 1988). Snuff dipping may lead to even higher levels of some types of adduct than to smoking (Carmella et al., 1990).

A variety of assays are available to determine carcinogen–macromolecular adducts in human tissues (La and Swenberg, 1996; Farmer and Shuker, 1999; Hecht, 1999; Lee et al., 1993; Wang et al., 2000). Although DNA adduct analysis is most commonly studied in relation to carcinogenesis, adducts also have been found in atherosclerotic lesions (Izzotti et al., 1995). Assay techniques include the phosphorus-32 (^{32}P)-postlabeling assay–nucleotide chromatography (Randerath et al., 1981; Phillips, 1997), immunoassays (Lee et al., 1993) fluorescence spectroscopy (Izzotti et al., 1991), gas chromatography–mass spectroscopy (GC–MS) (Farmer and Shuker, 1999; Hecht, 1999), and electrochemical detection (Park et al., 1989; Helbock et al., 1998). Each has its strengths and limitations, and almost all are challenged by low sensitivity and/or specificity. The less specific methods, such as the ^{32}P-postlabeling assay–nucleotide chromatography, when used as originally described (Randerath et al., 1981) or with modifications (Reddy and Randerath, 1986), offer the benefit of assessing exposure to complex mixtures because multiple adducts are measured at the same time. However, because the assay does not identify the types of adducts, any interpretations of the results are limited. Chemical specificity is helpful in assessing harm reduction products when the adducts are specific for tobacco (e.g., TSNAs or 4-aminobiphenyl in the absence of occupational exposure), whereas adduct assays that determine levels from endogenous sources (e.g., oxidative damage, methylation) are more difficult to use and interpret. The study of carcinogen–DNA adducts presents other challenges in interpretation; for example, carcinogen–DNA adduct levels are higher in the heart than in the lung (Randerath et al., 1989) while cancer is rare in the former. For the future, newer adduct methods may provide increased specificity and sensitivity, along with higher throughput.

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The use of target organ biomarkers can provide specific information about potentially carcinogenic effects and will best represent the biologically effective dose. Target organs include lung for lung diseases, oral mucosa for oral cavity diseases, bladder mucosa for bladder disease, and so forth. Surrogate markers that estimate levels in target organs, such as carcinogen–DNA adducts in blood, have been partially studied, indicating that blood levels might reflect target organ levels (Wiencke et al., 1995; Tang et al., 1995; Mustonen and Hemminki, 1992; Mustonen et al., 1993), but this is not yet firmly established. Protein (Meyer and Bechtold, 1996) and hemoglobin (Wang et al., 2000) adducts also may estimate levels of exposure at the target organ and thus be surrogates. Such assays offer technological advantages because these macromolecules are more abundant in blood than DNA, but the relationship of these other macromolecular adducts to DNA levels has been insufficiently studied.

A few studies show the decline of adducts following short-term and long-term smoking cessation. Most studies will necessarily rely on blood levels, and the half-life of adducts in blood will depend on the life span of various blood cell types. In humans, the half-life for 4-aminobiphenyl–hemoglobin adducts is 7-9 weeks, which is shorter than the life span of a red blood cell (Jahnke et al., 1990). PAH–DNA adducts in white blood cells have a half-life of 9 to 13 weeks (Mooney et al., 1995). In human lungs, it was reported that adducts persist in the lungs of ex-smokers (Randerath et al., 1989), but it is not known whether this is truly persistence or the formation of new adducts from the continuing presence of tobacco constituents such as PAHs or from other exposures such as diet or air pollution (Rothman et al., 1990).

Carcinogen–DNA adduct data have essentially not been used for population risk assessments. In one example, it was considered that a doubling of PAH–DNA adduct levels would result in an additional 2,400 cancer cases per million persons (van Delft et al., 1998), but the model assumed linear dose–responses; was not adjusted for age, gender, or race; and was too simplistic.

BIOMARKERS OF POTENTIAL HARM

These biomarkers reflect changes in a cell and its macromolecules that result from tobacco. These can range from isolated changes, with or without effects on function, to events that clearly lead to illness or are symptoms of the illness (i.e., cough). Examples of biomarkers of effect are provided in Table 11-8, which gives the reader a range of assays available but is not intended to be all inclusive.

Among the most promising biomarkers of effect for assessing harm reduction claims for cancer are those that measure DNA damage or alterations of genetic function (mutations, gross chromosomal changes, DNA methylation of promoter regions, etc.). While these biomarkers are envisioned for use in developing a molecular fingerprint reflecting a particular exposure, this has not occurred for tobacco carcinogens, and measurable effects thus far are relatively nonspecific. Nonetheless, a reduction in the level of genetic damage would logically be required if a tobacco-related PREP were to be successful in reducing cancer risk, although how much reduction of genetic damage would be needed to derive a benefit in terms of disease risk is unknown. Several types of assays are available. The main limitation today is that no assays have been shown convincingly to be sufficiently predictive of cancer risk. Chromosomal damage can be measured through classical cytogenetic alterations (Ramsey et al., 1995; Bender et al., 1988; Obe et al., 1982), micronuclei formation (Thorne et al., 1998), COMET (Speit and Hartmann, 1999; Poli et al., 1999), fluorescent in situ hybridization (FISH) (Ramsey et al., 1995; Pressl et al., 1999; van Diemen et al., 1995), or polymerase chain reaction (PCR) methods assessing loss of heterozygosity (using tandem repeats or comparative genomic hybridization) (Mao et al., 1997), where

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the latter two methods can be used for morphologically normal-appearing cells. Mutations in reporter genes, such as hypoxanthine phosphoribosyltransferase (HPRT) (Hou et al., 1999; Ammenheuser et al., 1997; Jones et al., 1993) or glycophorin A (GPA), have been used in blood cells, but it is better to identify mutation rates for cancer genes in biopsies from target organs or in surrogate tissues, and for genes such as p53 (Greenblatt et al., 1994) or KRAS (Scott et al., 1997; Lehman et al., 1996; Mills et al., 1995; Yakubovskaya et al., 1995). Although these assays are available, current technology limits their use in large scale epidemiological studies. The role of mitochondrial DNA lesions is receiving greater attention for cancer risk (Fliss et al., 2000), and the lesions associated with smoking might be useful (Liu et al., 1997). Among all the assays that have potential application to assessing harm reduction claims, only two studies have assessed prospectively the cancer predictive value of chromosomal aberrations (Hagmar et al., 1994; Bonassi et al., 1995), but they consisted of pooled heterogenous populations and were not focused on tobacco. Further studies are needed to indicate the value of these assays for determining harm reduction. Thus, none of these assays can be used today to allow claims of risk reduction, although in the proper setting they can suggest that such might occur.

Biomarkers of pathobiological effect include morphological markers of preneoplastic lesions (e.g., dysplasia), altered phenotypic expression of normal cellular functions (e.g., overexpression of the proto-oncogene Erb-B2), and mutations in cancer-related genes such as the p53 tumor suppressor gene. Some of these may be considered preclinical effects that are occurring before diagnosis. The lesions demonstrate a person's phenotype for exposure and predisposition that persist following DNA damage. Recent advances have made it possible to measure background mutations in cancer-associated genes of noncancerous tissues (Mao et al., 1997; Aguilar et al., 1994; Sidransky, 1997), which presumably are related to future cancer risk.

The study of mutations in the p53 tumor suppressor gene is uniquely suited for studying cancer etiology, exposure, and susceptibility (Harris and Hollstein, 1993), because p53 is involved in many cellular processes including maintenance of genomic stability, programmed cell death, DNA repair, and others (Soussi et al., 2000; Attardi and Jacks, 1999; Hollstein et al., 1999; Shimoda et al., 1994). The p53 gene, in particular, has a more frequent spectrum of mutations in tobacco-associated lung cancers (Bennett et al., 1999). An interactive effect of alcohol drinking and cigarette use in oral cavity and lung cancers leads to different types of p53 mutations (Brennan et al., 1995; Ahrendt et al., 1999, 2000). Interestingly, given that the p53 mutational spectrum for lung cancer is similar worldwide (Hartmann et al., 1997), it is likely that tobacco smoke is the major determinant of lung p53 mutations worldwide. Evidence for a relationship of gene–environment interactions and mutation risk in the p53 gene can be found from a Japanese study of *CYP1A1* (Kawajiri et al., 1996), where a fivefold increase in risk of p53 mutations was found for smokers with lung cancer and the "at-risk" genetic variant. This risk increased further for persons who also lacked the glutathione *S*-transferase (*GSTM1*) gene. In one study from Norway, smokers with lung cancer who lacked *GSTM1* also had more p53 mutations, especially transversions (Ryberg et al., 1994). For oropharyngeal tumors, the frequency of p53 mutations was increased for the same *CYP1A1* variant allele (Lazarus et al., 1998). An increased risk for p53 mutations in lung cancer also has been found in Japanese persons with less common variants of *CYP2E1* (Oyama et al., 1997).

Newly developed technologies allow for the detection of loss of heterozygosity (LOH) in small amounts of tissue. Losses at chromosome 3p14, 9p21, and 17p13 have been seen in the lungs of both smokers and former smokers, where the first is less frequent in former smokers than current smokers (Mao et al., 1997).

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An important area that has not been well studied is the effect of tobacco toxicants on the induction of enzymes that might affect cancer risk. For example, cytochrome P-450 enzymes are induced with tobacco smoking (e.g., aryl hydrocarbon hydroxylases [AHHs]) (Guengerich, 2000; Bartsch et al., 1995; Nakajima et al., 1995; McLemore et al., 1990; Bartsch et al., 1991 Rojas et al., 1992). Induction is related to greater amounts of DNA damage (Bartsch et al., 1991; Geneste et al., 1991). It remains to be tested whether a tobacco-related PREP can reduce AHH exposure so that other carcinogenic exposures will be less harmful. Many proteins are induced in relation to DNA damage (e.g., p53) (Bjelogrlic et al., 1994). Whether higher levels of these proteins increase or decrease the risk of disease remains unknown.

Several biomarkers can be studied in relation to cardiovascular disease risk, but none of these are specific to tobacco smoking, such as blood lipid level (Wald et al., 1989; Hellerstein et al., 1994; Freeman et al., 1993; Ludviksdottir et al., 1999; Cullen et al., 1997; Stubbe et al., 1982), which changes with cessation (Green and Harari, 1995), or urinary excretion of thromboxane A_2 metabolites (Wennmalm et al., 1991; Nowak et al., 1987; Rangemark et al., 1992; Lassila et al., 1988). F_2-Isoprostanes in blood have a dose–response relationship to smoking (Morrow et al., 1995). Tobacco smoking is associated with decreased weight (Green and Harari, 1995) and therefore modifies the relationship of weight gain to increased risk of heart disease (Fulton and Shekelle, 1997). Blood pressure has been studied but is not clearly associated with smoking (Green and Harari, 1995) Other biomarkers that have been suggested to reflect an increased cardiac disease risk include reduced platelet survival (Fuster et al., 1981). Newer imaging methods such as electron-beam computed tomography (O'Malley et al., 2000; Raggi et al., 2000) are being used to assess heart disease risk, and these methods might be used to assess the decreasing rate of formation of atherosclerosis or calcium when using a PREP.

Biomarkers of developing respiratory illness have been assessed in different ways, and several studies have specifically assessed the effects of smoking reduction separately from cessation. Symptoms, albeit late effects, such as cough, chronic phlegm production, wheezing, and shortness of breath have been used and improve with smoking cessation (Kanner et al., 1999; Buist et al., 1976). Reducing smoking, without quitting, also is associated with a reduction in symptoms (Buist et al., 1976). There are many studies that explore decrements of pulmonary function related to cigarette smoking. While such decrements occur with aging independent of smoking, further decrements are induced by smoking (Lange et al., 1989; McCarthy et al., 1976). Declines in the forced expiratory volume at 1 second (FEV_1) are associated with increased disease and mortality, including nonpulmonary diseases (James et al., 1999). The decline in pulmonary function tests slows with complete cessation (Kanner et al., 1999; Buist et al., 1976; Lange et al., 1989; McCarthy et al., 1976) and with greater than 25% reduction in the number of cigarettes smoked per day (Buist et al., 1976; Lange et al., 1989; McCarthy et al., 1976). Smoking reduction in the elderly apparently showed no effect in slowing the rate of decline (Lange et al., 1989). Bronchioalveolar lavage has been used, although it is invasive, and different types of assays can assess inflammation, neutrophil elastase α_1-antiprotease complex, and α_1-antitrypsin (Rennard et al., 1990). Induction of these components reverses with smoking reduction (Rennard et al., 1990), and some markers such as alveolar neutrophils, neutrophil elastase α_1-antiprotease complexes, and alveolar macrophages decrease in smokers who reduce their amount of smoking when provided with nicotine replacement therapy (NRT) (Rennard et al., 1990).

Several nonspecific biomarkers of effect are related to smoking, such as leukocyte count (Wald et al., 1989; Phillips et al., 1992; Sunyer et al., 1996; Parry et al., 1997), which reverses with cessation (Green and Harari, 1995; Sunyer et al., 1996) and then increases again with re-

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sumption of smoking (Sunyer et al., 1996). Levels remain increased to some extent in former smokers compared to never smokers (Parry et al., 1997). Whether these findings, however, are independent predictors of disease risk has had limited study (e.g., mortality) (James et al., 1999), and the differences that can be found may be due to disease unrelated to smoking (Wald et al., 1989). Some of these parameters are covariates (James et al., 1999). Thus, such markers would be less useful for assessing harm reduction claims but might be useful for assessing exposure reduction claims.

There are several short-term effects on the body that can be considered both from the perspective of disease and as a biomarker of effect. Examples include periodontal disease, abnormal glucose tolerance tests, and decreased birthweight of infants born to mothers who smoke. Also, changes in adult body weight can be measured in the context of harm reduction. It is well known that smoking increases metabolism and decreases appetite, while stopping smoking is associated with weight gain (O'Hara et al., 1998). This can be a very important marker of smoking effects since the consideration of weight is often a factor in persons' beginning smoking or resisting cessation.

HOST SUSCEPTIBILITY

Host susceptibility could modify the risk of tobacco-related disease and, therefore, the effects of PREPs. Host susceptibility can be influenced by genetic susceptibility, age, gender, ethnicity, health status, and so forth. These will not be discussed in detail except for genetic susceptibility, but any relevant potential modifying factor should be considered in the assessment of a PREP.

The study of genetic susceptibilities can improve the accuracy of estimates of disease associations (Khoury and Wagener, 1995). Tobacco toxicants affect people to variable degrees. It is therefore reasonable to assume that harm reduction strategies would affect people differently. There is large interindividual variation in cellular responses—for example, in metabolism and detoxification of toxicants and DNA repair. As other cellular responses to DNA damage are identified (e.g., cell-cycle delays, heat shock), interindividual variation in risk is likely to be discovered for these as well. Interindividual effects in cellular responses could be due to genetically determined enzyme expression, kinetics, or stability. Also, induction of enzymes from previous exposures or comorbidity also may contribute to cancer risk, and induction has a genetic component.

Susceptibility to disease from genetic variability can range from small to large, depending on the genetic penetrance. Highly-penetrant cancer susceptibility genes cause familial cancers but account for less than 1% of all cancers (Fearon, 1997). Low-penetrant genes cause common sporadic cancers and can have great public health consequences (Shields and Harris, 2000).

Genetic susceptibility can be assessed either phenotypically (measuring the resultant enzymatic function) or genotypically (determining the genetic code). Examples are provided in Table 11-9. Phenotypic assays may include determining enzymatic activity by administering probe drugs to individuals and measuring blood levels or urinary metabolites, assessing carcinogen metabolic capacity in cultured lymphocytes, or establishing the ratios of endogenously produced substances such as estrogen metabolite ratios. One extensively studied phenotype in relation to smoking risk is AHH activity (Kellermann et al., 1973; Kouri et al., 1982). In general, it is preferable to use a gene-based assay to assess disease risk because DNA is easier to obtain and the assays are technically simpler. However, phenotypes usually represent a multigenic trait, which may not be adequately characterized by only one genetic assay. Therefore, there is a role for both gene- and phenotype-based assays in research studies and PREP assessments. Examples of fre-

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quently studied genetic polymorphisms in tobacco-related cancers that have been shown in some studies to modify smoking-related disease risk include the *N*-acetyltransferase 2 (*NAT2*) (Brockmoller et al., 1996, 1998; Henning et al., 1999), glutathione *S*-transferase M1 (*GSTM1*) (Cullen et al., 1997; Brockmoller et al., 1996, 1998; Rebbeck, 1997; Jourenkova-Mironova et al., 1999; Jourenkova et al., 1998; Bell et al., 1993), cytochrome P-450 1A1 (*CYP1A1*) genes (Ishibe et al., 1997; Bishop, 1987), glutathione *S*-transferase Pi (Ryberg et al., 1997), and others (Jourenkova-Mironova et al., 1999; Wiencke et al., 1997; Rosvold et al., 1995). These and other genetic polymorphisms are believed to affect levels of biomarkers, such as DNA adducts (Kato et al., 1995; Ryberg et al., 1997; Pastorelli et al., 1998; Yu et al., 1995).

In the general population, DNA repair capacity decreases in humans with aging (Wei et al., 1993; Liu et al., 1994), which would make this an acquired risk factor for cancer and might explain a portion of the increased cancer risk in the elderly (Simpson, 1997). Both genotyping and phenotyping assays for DNA repair or cell-cycle control that affects DNA repair might be useful in identifying individuals who might benefit from harm reduction strategies. Tobacco toxicants can affect DNA repair (Grafstrom et al., 1994), so that the effects of both tobacco toxicants and heritable capacity on DNA repair can be considered in assessing harm reduction products. It should be noted that cigarette smoking induces levels of some repair enzymes (Hall et al., 1993; Slupphaug et al., 1992; Drin et al., 1994), so caution must be used for some phenotyping assays.

Inherited susceptibilities via specific genetic polymorphisms that affect the efficiency of DNA repair (e.g., for base excision repair) have been identified recently (Mohrenweiser and Jones, 1998). Studies now being completed indicate an effect of these genetic variants on tobacco-related cancer risk (Sumida et al., 1998), some of which have functional effects on DNA repair (Lunn et al., 2000, 1999). A nonspecific DNA repair assay, which measures chromosomal aberrations in human cultured lymphocytes after an in vitro challenge with a mutagen, has shown initial promise. In this case, an increased mutagen-related aberration rate has been observed in persons with primary and secondary upper aerodigestive tract cancers (Cloos et al., 1996), multiple primary cancers (Cloos et al., 1994), and lung cancer (Spitz et al., 1995; Li et al., 1996; Wei et al., 1996).

Genetic susceptibilities for genes other than those involved in carcinogen metabolism and DNA repair are also being investigated (Jin et al., 1995; Sjalander et al., 1996). There has been less study of genetic susceptibilities for coronary artery disease (Gealy et al, 1999). It is likely that these genes also will play a role in modifying disease risk (see Chapter 13).

GENETIC PREDISPOSITIONS TO SMOKING ADDICTION

The greatest contributors to smoking addiction are the availability of tobacco and cultural acceptance of tobacco smoking. Genetics plays a lesser role. The tobacco smoking epidemic has occurred only over the last 50 to 70 years, and it is unlikely that human genetics have evolved in that amount of time. Nonetheless, twin studies indicate a genetic role for both smoking initiation and smoking persistence (Carmelli et al., 1992; Heath et al., 1993a,b).

People smoke in ways that will maintain a desired blood nicotine level. Nicotine in turn stimulates reward mechanisms in the brain. Presynaptic nicotinic acetylcholine receptors stimulate the secretion of dopamine into neuronal synapses. There also are effects on other pathways, such as those that involve serotonin. For dopamine, synaptic dopamine stimulates dopamine receptors; five subtypes have been identified, which are considered to be D_1- or D_2-like. Synaptic dopamine levels are governed by presynaptic release and the presynaptic dopamine transporter protein. In humans, there are different types of data supporting the link between nicotine and do-

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pamine. Nicotine self-administration through tobacco smoking may reduce the adverse consequences of Parkinson's disease, attention deficit disorder, and schizophrenia (Seeman, 1995; Olincy et al., 1997; Bannon et al., 1995), diseases thought to be related to dopamine abnormalities. Also, smoking probably relieves depression (Gilbert and Gilbert, 1995), and the dopamine transporter inhibitor antidepressants (e.g., bupropion SR) are now used to treat nicotine addiction (Hurt et al., 1997; Jorenby et al., 1999).

The genes that code for dopamine receptors (e.g., *DRD2, DRD4*), dopamine transporter reuptake (*SL6A3*), and dopamine synthesis (e.g., dopamine hydroxylase, tyrosine hydroxylase, tryptophan hydroxylase, catechol-*O*-methyltransferase, monoamine oxidase) are polymorphic. Some of the polymorphisms result in altered protein function. Persons with higher levels of synaptic dopamine, or "more stimulation" of dopamine receptors may have less rewarding effects of nicotine and so would be less likely to become smokers and would more easily quit. For example, in a study of 500 smokers and nonsmokers, several candidate genes have been implicated (Shields et al., 1998; Lerman et al., 1998 and 1999), whereas other studies of candidate genes have yielded null results (Lerman et al., 1997). Other investigators also have reported supporting evidence (Noble et al., 1994; Comings et al., 1996; Spitz et al., 1998). Thus, it is likely that there is a genetic contribution to smoking addiction and behavior and there may also be a genetic influence on who benefits from PREPs.

BIOMARKER ASSESSMENT FOR ENVIRONMENTAL TOBACCO SMOKE EXPOSURE

Biomarker assessments in persons exposed to environmental tobacco smoke (ETS) are problematic because exposures occur at much lower levels than in smokers, and therefore the level of detection is limiting (Benowitz, 1999). The most consistently used biomarkers are those that reflect exposures, namely cotinine (serum, plasma, or urine), rather than biologically effective doses or biomarkers of effect. Such biomarkers, for example, can show that adolescents are exposed to tobacco smoke through household smoking (Bono et al., 1996). Urinary metabolites of tobacco-specific nitrosamines also have been found in persons exposed passively to smoke (Atawodi et al, 1998; Hecht et al., 1993; Parsons et al., 1998). DNA adducts in the lung are also detected in persons who are thought to be nonsmokers (Kato et al., 1995). Children exposed to modest levels of ETS have been found to have increased concentrates of 4-aminobiohenyl adducts of PAH-albumin adducts (Tang et al., 1999). Although it may follow that proven methods to reduce harm in smokers would apply to nonsmokers with passive exposure, there are circumstances in which passive smoke exposure might be substantial (e.g., cigar smoking).

DEVELOPMENT AND VALIDATION OF BIOMARKER ASSAYS, INCLUDING QUALITY CONTROL

The use of biomarkers in assessing harm reduction can be helpful only when the assays have undergone rigorous development and validation. Reliance on insufficiently validated biomarkers becomes problematic because they are of uncertain value and so should not be used to support a claim of exposure or risk reduction. The design and development of a biomarker assay must conform to the original goals—that is, the assay should have sufficient specificity, it should be quantitatively reproducible in humans at the levels that occur when exposure reduction is achieved, and other assays should be available to corroborate the qualitative and quantitative results. Many pitfalls have already been found in biomarker development. There are examples of

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biomarker assays that are more difficult to perform at levels observed in humans compared to the use of higher-level laboratory chemical standards (e.g., immunoassays) (Santella et al., 1988). Some methodologies can artifactually affect assay results (e.g., introduction of oxidative damage) (Farmer and Shuker, 1999). In some cases, measurements of in vivo formation can be skewed by exogenous exposure to the biomarker (e.g., dietary ingestion of 3-alkyladenine) (Prevost and Shuker, 1996).

Validation of a biomarker assay includes a determination of replicability (e.g., coefficient of variation), interobserver and interlaboratory variability, intraindividual variation, and interindividual variation. These validation steps must be done using known controls that simulate human exposure levels and harm. Thus, the assay should be validated in light and heavy smokers, former smokers, and never smokers. Caution must be used in interpreting assay results in the context of certain study designs. For example, the reliability of biomarkers thought to be related to disease risk in case-control studies is problematic for markers that might be affected by disease status (differential case bias) (Wald et al., 1989).

Research laboratories providing data that can impact individual or public health should have adequate quality control and quality assurance procedures in place. The definition of adequate will depend on the population under study and the number of subjects. In clinical pathology laboratories, standards and protocols have been established by organizations such as the College of American Pathologists and the National Committee for Clinical Laboratory Standards. In a research laboratory that performs biomarkers studies assessing PREPs, there should be standards for proficiency testing, quality improvement, quality control, use of standards, methods for interpretation, specimen handling, specimen labeling, specimen processing, and reporting of results. There also should be criteria for facility and equipment maintenance.

CONCLUSIONS

The assessment of a PREP will have to consider external exposure and markers of internal exposure, estimates of the biologically effective dose, and biomarkers of potential harm. A risk reduction claim should be based on disease reduction, but time limitations mandate the use of biomarkers for both exposure and risk reduction assessments. Measurements of the number of cigarettes per day, smoking duration, estimated lifetime exposure, smoking topography, and so forth, provide an effective indicator of exposure that has been associated with risk. However, these measures may be insensitive to small changes in risk, are difficult to assess accurately over time, and have not been tested in the context of harm reduction. Also, because there is interindividual variation in how the body responds to these exposures, such measures might not be sufficiently accurate for new products intended to decrease exposure. The relationship of external exposure markers to disease risk might be less predictable for new products. Currently, there is sufficient evidence to show that biomarkers can provide better estimates of risk in the context of exposure, and therefore they will likely be able to provide improved assessments for harm reduction products. However, no single biomarker has been sufficiently validated and related to disease risk to be recommended as an intermediate biomarker of cancer risk. Thus, different types of biomarkers along the pathway from internal exposure to biologically effective dose, and to potential harm are needed, and additional research is necessary to identify the best combination of markers to be used. Experimental toxicity testing (in vitro and animal models) are not sufficient to support a tobacco-related PREP claim because only biomarkers can show that the PREP reduces exposure adequately enough to imply risk reduction. However, the use of intermediate biomarkers as surrogate risk factors for disease may overestimate the number of persons

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who actually develop disease because not all early changes in morphology or function progress to disease. On the other hand, it may underestimate if, as expected, other mechanisms are involved in the disease process that are not reflected by the biomarkers. Therefore, the implication of a potential benefit in a harm reduction strategy could also be an overestimate, but this limitation in the scientific methodology for identifying sufficiently specific biomarkers of risk requires acceptance at the current time.

Previously, the most common way in which exposure reduction has been inferred is through the use of methods that simulate human smoking behavior, such as the FTC method. Although they provide a standardized way to assess cigarettes, it is clear that these methods have limited usefulness because people smoke their cigarettes differently than the machine, with resultant differences in the types and amounts of exposure.

The use of biomarkers improves exposure assessments (e.g., characterizing low-dose exposures or low-risk populations), provides a relative contribution of individual chemical carcinogens from complex mixtures (e.g., TSNAs and PAHs in cigarette smoke), and estimates total burden of a particular exposure where there are numerous sources (e.g., BaP from air, tobacco, diet, and occupation) (Vineis and Porta, 1996). Biomarkers also can establish differences in individual susceptibilities and whether there are differences in response depending on dose. Thus, biomarkers that measure both complex exposures and single tobacco product constituents are needed and should be assessed for the range of possible human exposures and those that assess complex exposures should carry a greater weight. Also, some biomarkers should be used that are less specific for individual tobacco constituents in order to monitor for the introduction of new hazards from tobacco-related PREPs.

Today, there remain technical limitations to the use of biomarkers. Depending on the harmful effect, surrogate assays in nontarget fluids or organs that represent effects in target organs may be easier to perform in humans because the target tissues might not be easily accessible. However, if such is the case, the relevance of the surrogate biomarker to the effect in the target organ should be demonstrated.

The use of a biomarker for harm reduction assessment should include several considerations, including where it is along the pathway from exposure to disease, its specificity and sensitivity, available harm dose–response data, available reduction in harm dose–response data, target tissue effect, and how it is validated. The need for validation cannot be overemphasized. Each biomarker should be validated for its relationship to exposure and harm and also as a laboratory assay that provides reliable and reproducible data. Separately, the way a biomarker is affected by interindividual variation in response and by behavior should also be considered.

Assessment of harm and harm reduction should be made through direct human experience, as the products are used by the general population. Most of what is known about harmful tobacco products has resulted from epidemiology, supported by in vitro studies, laboratory animal studies, and human experiments. However, while epidemiological studies can provide the most definitive data about tobacco harm and harm reduction products, the study of diseases with long latency (e.g., cancer, heart disease, chronic obstructive pulmonary disease) is problematic because such studies require many years before they provide useful data. Thus, because definitive evidence for a new risk reduction product is not available short-term markers that reflect long-term outcomes are needed. If an approach for assessing risk reduction products required only epidemiological data measuring disease outcome prior to use by the public, then an opportunity to reduce morbidity and early mortality might be missed. However, the use of intermediate

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markers does not replace long-term follow-up and epidemiological surveillance, but allows judgments to be made until such data are forthcoming.

Biomarkers of internal exposure, biologically effective dose, or potential harm have been validated to different degrees. It is typically easier to show a direct relationship of external exposure to biomarkers in the following order: internal exposure, biologically effective dose, and potential harm. Conversely, it is typically easier to show a direct relationship of disease outcome to biomarkers in the following order: potential harm, biologically effective dose, and internal exposure. It might be acceptable to rely on external exposure measurements for considering risk and dose–response, but only with substantial corroborative biomarker data. The best strategy for assessing the claims for risk reduction methods is with several markers that range from exposure to outcome, one being linked to another, and at least one with which a dose–response risk assessment can be made.

The recommendation that harm reduction products should be assessed with the use of biomarkers reflects sufficient available data to show that the public is composed of individuals with different cultural and heritable traits that affect how people use tobacco products and respond to them. To achieve the best confidence that a PREP will reduce risks for persons who cannot stop smoking, both well-validated methods for predicting risk, including external exposure indicators, and the best available biomarker assays should be used.

RESEARCH AGENDA

There are currently different methodologies for assessing PREPs, but substantial research is needed to increase confidence in the application of these methods. Although it may be possible to improve external methods for assessing exposures, such as through modification of the FTC method or improving questionnaire assessments, there is so much variability in human smoking behavior that it is believed these methods could never be much more helpful than they already are. This recommendation does not imply that questionnaires and topography instruments are not helpful in assessing smoking behavior, because they are, but it is unlikely that the methodology can be improved substantially. Indeed, clinical epidemiological studies generally have to integrate more variables for smoking behavior (e.g., accurately documenting changes in smoking, brand switching).

The development and validation of biomarkers for assessing harm reduction must be accelerated for all diseases, especially for cardiovascular and respiratory diseases because less research has been conducted compared to cancer.

The use of a biomarker for assessing harm reduction should be considered using the criteria provided in this chapter. Dose–response relationships should be established, and the biomarker should be assessed for reversibility in smoking cessation trials. In all studies of biomarker validation, consideration should be made of what nontobacco exposures, if any, would influence the biomarker study results. Also, biomarkers have to be tested and validated in different populations, to determine whether they are affected by susceptible subpopulations, and within genders, races, or ethnicities. Research efforts should focus on biomarkers that might be used for existing cohort studies, where disease outcome already is known. For example, markers are needed that can be used in serum or small amounts of DNA from stored samples. This is the best way to identify a relationship between exposure, a biomarker, and disease risk. Substantial research is needed to identify the relationships between biomarkers to exposure, biologically effective doses, and biomarkers of harm. Study designs that can provide these linkages are needed, and the best evidence will come from cohort studies.

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Internal biomarkers of exposure such as cotinine, nicotine boosts, carbon monoxide (CO), and CO boosts provide good information about exposure, including to environmental tobacco smoke, but additional markers, such as urinary anabasine and anatabine levels, have to be developed for use in persons who are concurrently using nicotine replacement therapy. Increased efforts to measure urinary excretion of carcinogen metabolites, which are currently showing promise for use in risk assessment of active smoking and ETS, are needed. Examples include urinary excretion of tobacco-specific nitrosamines and polycyclic aromatic hydrocarbons and urinary mutagenicity, where these reflect both single and complex markers of exposure, respectively. Also, markers with longer half-lives would be useful to avoid confounding by recent changes in smoking behavior.

Biomarkers that reflect the biologically effective doses of exposure to carcinogens must be improved and validated. Newer technologies are now available that are more sensitive (e.g., mass spectroscopy) and can provide more information, and these should be applied in experimental systems and human studies that were developed long before such methods were available. For example, the determination of carcinogen–DNA adducts might be useful where small amounts of tissue are available (e.g., buccal swabs, sputum, blood).

More biomarkers of potential harm are currently being developed than any other types. This is because pathobiological pathways are well understood and newer technologies are available to explore them. However, along with better technologies will come limitations in the interpretation of new data (e.g., mRNA expression assays, proteomics). As researchers explore greater numbers of gene–smoking interactions and accumulate data for numerous genes expressed in response to exposures, it is clear that there are insufficient methods to analyze data where there are a substantial number of predictor variables. Also, some data will have to be reduced to clusters or other smaller units that are understandable in the context of biological hypotheses. Increased research is needed in methodologies to interpret these types of data, to validate the new models in the context of disease outcome.

For cancer, increased efforts are needed to assess target organ assays, such as genetic damage in lung cells in sputum and exfoliated bladder cells in urine, in persons years before they have a clinically detectable cancer. Given that genetic damage is only one part of the carcinogenic process, additional efforts are necessary to develop biomarkers for other pathways, such as gene silencing through hypermethylation of promoter regions. For cardiac disease, additional studies are needed to validate biomarkers of platelet function, endothelial function, endothelial thickening, and plaque formation and thrombosis. For respiratory disease, better markers are needed to assess changes in lung function that predict chronic obstructive pulmonary disease and asthma, and to assess immunological changes that will increase risk of respiratory infections.

It would be optimal to identify biomarkers that can be used to assess risk for several diseases. For example, biomarkers of oxidative damage might identify risk for cardiac disease, cancer, and respiratory illness. However, because the relationship of oxidative damage to these diseases remains mostly an unproved hypothesis, research is needed in this area.

Biomarkers will have to assess PREPs for single tobacco constituents and complex mixtures. The use of biomarkers that can assess multiple exposures from complex mixtures is critical because new tobacco-related PREPs might include compounds that are not present in existing tobacco constituents, or the ratio of exposures to individual constituents might change. A committee of experts should be convened to consider and identify those biomarkers that have the most promise and to determine what combination of biomarkers should be part of a panel for assessing PREPs.

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To identify those biomarkers most useful for assessing harm reduction products, current efforts have to be focused on clinical trials that assess the effects of switching brands, using new products, and reducing daily consumption of tobacco through the concomitant use of nicotine replace therapy or other aids used for smoking cessation.

There are unique opportunities in epidemiological studies to validate biomarkers for use in assessing harm reduction strategies. Specifically, cohorts of participants in smoking cessation programs and former smokers should be established because these individuals represent the best possible reduction in the risk due to smoking. The collection of tissues and fluids from persons who have quit smoking and comparisons of persons who do and do not develop disease would be very helpful in determining which biomarkers have the most predictive value. This should be done in the context of previous smoking history to identify which persons would obtain the greatest benefit from cessation and how biomarkers might be able to identify individuals at greatest risk within these groups. Some diseases, such as cardiovascular disease, have a relatively rapid decline in risk following cessation so it would be quicker to validate cardiac disease risk factors. For cancer, the studies will take much longer. Monitoring populations that are at the highest risk of cancer, such as persons with resected early-stage lung cancer or bladder cancer, might be useful in this context. If a biomarker cannot predict increased risk in former smokers, it is unlikely to be useful in assessing PREPs.

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Table 11-1 Exposure and Biomarker Assessment Definitions	
Exposure or Biomarker Assessment[a]	**Definition**
External Exposure Marker	A tobacco constituent or product that may reach or is at the portal of entry to the body
Biomarker of Exposure	A tobacco constituent or metabolite that is measured in a biological fluid or tissue that has the potential to interact with a biological macromolecule; sometimes considered a measure of internal dose
Biologically Effective Dose (BED)	The amount that a tobacco constituent or metabolite binds to or alters a macromolecule; estimates of the BED might be performed in surrogate tissues
Biomarker of Potential Harm	A measurement of an effect due to exposure; these include early biological effects, alterations in morphology, structure, or function, and clinical symptoms consistent with harm; also includes "preclinical changes"

[a]Categories and definitions reflect concept that the critical exposure is at the level of a biological macromolecule, so that exposure for this discussion is not limited to a measurement at the portal of entry to the body.

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Table 11-2 Measurements Used For Assessing Harm Reduction Products

Factor	Comment
Type of measurement	Types of measurements that can be used include external exposure assessment, biomarkers of exposure, biomarkers that represent the biologically effective dose, and biomarkers of potential harm. Depending on the context, the PREP, and the outcome of interest, different measurements might be more appropriate, although it is likely that a combination will be needed
Target tissue and outcome effect	Is the measurement used for detecting effects in target or surrogate tissues, and is this a measurement of pathogenesis?
Dose–response data	Measurements must have a dose–response relationship that is understood on a mechanistic basis. Biomarker should be able to demonstrate effects from exposure over the range of human experience, so that it can show exposure reduction from a PREP
Harm reduction in dose–response data	Biomarker should be able to predict a decrease in disease incidence after exposure is reduced
Specificity	Is the measurement specific for a tobacco product constituent, or does is also measure exposures from nontobacco products?
Sensitivity	Is the measurement sensitive enough to measure what it is supposed to measure in humans within the possible exposure ranges?
Validation	Are there sufficient data to show that the assay is reproducible?

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Table 11-3 Methods for PREP Assessment

Category	Type of Measurement	Target vs. Surrogate	Examples	Strengths	Limitations
External exposure	External exposure assessment	Neither	Questionnaire data, FTC yield	Inexpensive	Does not reflect actual internal doses
Biomarker of exposure	Internal dose	Target tissue	Polycyclic aromatic hydrocarbon in lung tissue	Provides integrated measure of external exposure and smoking behavior	Expensive; may not be specific for tobacco products; does not necessarily reflect biologically effective dose; tissue may be difficult to access; may be difficult to validate as a risk marker for disease
		Surrogate tissue	Urinary measurement of tobacco constituent or metabolite, exhaled CO, carboxyhemoglobin, urinary mutagenicity	Easily accessible; provides integrated measure of external exposure and smoking behavior; metabolites reflect host capacity for metabolism and clearance	May not be specific for tobacco products; does not necessarily reflect biologically effective dose; may be difficult to validate as a risk marker for disease
Biologically effective dose	Biologically effective dose	Target tissue	Carcinogen–DNA adducts in human lung tissue, exfoliated bladder cells, or oral mucosa	Reflects integrated measure of external exposure, smoking behavior, metabolic activation, DNA repair capacity, cell-cycle control, and capacity for apoptosis	Difficult to measure and validate as a disease risk marker, predictive value for disease risk is insufficiently studied, more commonly reflects internal dose to a target macromolecule rather than disease risk
		Surrogate tissue	Carcinogen–DNA or hemoglobin adducts; DNA adducts; lipid peroxidation	Does not require invasive procedures, greater amount of tissue is generally available; more likely to be used in an epidemiological setting	Relationship to disease risk is not fully established
Biomarker of potential harm	Early biological and genetic effects	Target tissue	Changes in RNA or protein expression, somatic mutations, and LOH in normally or abnormally appearing tissue; change in methylation or gene control; mitochondrial mutations, mRNA expression arrays, or proteomics	Assessment of mechanistic pathway leading to disease	Tissue difficult to obtain; technically difficult; relationship to disease risk difficult to establish; harmful effects may already be present; bioinformatics with which to process information not yet available
	Alterations in morphology, structure, or function	Target tissue	Osteoporosis, hypertension, cough, hyperplasia, dysplasia, lipids, blood coagulant pathways, mRNA expression arrays, or proteomics	Greater ability to identify risk for disease with marker	Tissue difficult to obtain; late effects where harm has already occurred; bioinformatics with which to process information not yet available
	Surrogate assays	Surrogate tissue	Leukocytosis; HPRT mutations; chromosomal aberrations; circulating lymphocytes; mRNA or protein expression via microarrays in cultured blood cells	Easily accessible; provides integrated measure of external exposure and smoking behavior; metabolites reflect host capacity for metabolism and clearance	Relationship to target organ effect is difficult to prove; specificity for tobacco product needs to be proved; bioinformatics not yet available
Effect modifiers	Measures of interindividual variation	Neither	Genetic polymorphisms for genes involved in disease pathways	Reflects lifetime response to exposure; high throughput possible	Candidate gene approach will typically study many polymorphisms that are not related to disease risk
		Target	Enzyme induction of metabolizing enzymes	Integrated assessment of how prior exposures or genetic traits affect exposures and harm	Tissue technically difficult to obtain; laboratory validation difficult

NOTE: HPRT = hypoxanthine phosphoribosyltransferase; LOH = loss of heterozygosity.

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Source: https://www.industrydocuments.ucsf.edu/docs/jnlx0001

Table 11-4 External Exposure Assessment[a]

Category	Variables Used in Literature	Related to a Disease Outcome[b]	Strengths	Limitations
FTC machine method	Tar yield Nicotine yield Individual smoke constituent yield	Yes	Standardized method for yields	Little relationship to actual human experience
Subject smoking history	Cigarettes per day Years of smoking Age of initiation Recall of inhalation depth Usual type of cigarette smoked Quitting attempts Cumulative tar exposure	Yes	Inexpensive assessment; generally considered reliable, except in some circumstances listed in limitations	Recall is subject to self-perceptions of risk. Reporting is variable depending on context, such as smoking cessation program or where recall bias might exist in epidemiology studies. Known limitations for persons who are switching brands or altering smoking behavior; also not sufficiently reliable in smoking cessation studies. Thus, not sufficiently reliable in harm reduction studies
Smoking Topography	Puff duration Puffs per cigarette Interpuff interval Puff volume	No	Direct measure of inhalation exposure per cigarette. Can be used to assess effects of cigarette brand switching	Measurement performed in artificial environment

[a]References are not provided in this table but can be found in the text of this and disease-related chapters.
[b]Any report related to a disease outcome where the report is plausible but has not necessarily been replicated.

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Source: https://www.industrydocuments.ucsf.edu/docs/jnlx0001

Table 11-5 Major Tobacco Use Surveys

Survey	Sponsor	Population	Size	Setting	Comments
National Health Interview Survey (NHIS)	National Center for Health Statistics, Centers for Disease Control and Prevention (CDC)	Civilian, noninstitutionalized adults over age 18; children by proxy	More than 38,000 families in 1998	Household interview with responses typed directly in laptop computer; annual	Excludes homeless not in shelters, military personnel, prisoners, hospital patients Data: cigarette, chewing tobacco, cigar, and pipe use since 1965 Oversampling of black American and Hispanic populations
Behavioral Risk Factor Surveillance System (BRFSS)	CDC and individual states	Noninstitutionalized adults over age 18		Computer-assisted telephone interviews; annual	Added smokeless tobacco use questions in 1987 State level
National Health and Nutrition Examination Survey (NHANES)	CDC	Age 2 and over	Approximately 40,000 participants between 1988 and 1994	Personal interview with physical exam and blood tests; periodic	Serum cotinine measurements Oversampling of children 1–5years, adults over age 60, black Americans, and Mexican Americans
National Household Survey on Drug Abuse (NHSDA)	National Institute on Drug Abuse and Substance Abuse and Mental Health Services Administration	Noninstitutionalized civilian population over age 12	Approximately 25,500 participants in 1998	Household interview; self-administered through a computer; annual	State level Oversampling of black Americans, Hispanic Americans, and youth
American Legacy Foundation Survey		Sixth to twelfth grade students		School based	Evaluates knowledge of and attitudes towards all forms of tobacco, including bidis and Kreteks
Monitoring the Future Survey (previously, the National High School Senior Survey)	University of Michigan Survey Research Center	Eighth, tenth, and twelfth grade students	Approximately 50,000 students from public and private high schools	Classroom based; self-administered; annual	Random sample from each senior class is followed after graduation for longitudinal data
Youth Risk Behavior Surveillance System (YRBSS)	CDC	Ninth to twelfth grade students		Classroom based; self-administered; biennial	Over sampling of black and Hispanic-American students Combination of national, state, and local surveys

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Source: https://www.industrydocuments.ucsf.edu/docs/jnlx0001

Table 11-6 Biomarkers of Exposure[a,b]

Category	Variables Used in Literature	Dose-Response Data Available	Associated with Cessation or Half-life	Chemical Specificity	Specific to Tobacco	Related to a Disease Risk[c]	Strengths	Limitations
Nicotine-related biomarkers	Nicotine	Yes	2 hr	Yes	Yes (except when using nicotine replacement therapy [NRT])	Yes (addiction only)	Direct measure of exposure	Short half-life dependent on a person's ability to metabolize nicotine and time of sampling. Not useful with concurrent use of NRT.
	Nicotine boost (pre- and post-cigarette nicotine levels)	Yes	NA	Yes	Yes	NDA	Measures exposure to single cigarette	Requires two blood draws. Short-term marker only
	Cotinine	Yes	17 hr	Yes	Yes (except when using NRT)	Yes (addiction only)	Well validated; can be measured easily in urine, plasma saliva, or hair. Useful for environmental tobacco smoke	Short-term marker only. At higher levels of smoking, dose-response relationship is less clear and there is wide overlap among smokers
	Other nicotine metabolites	Yes	Depends on metabolite	Yes	Yes (except when using NRT)	NDA	Allows for assessment of nicotine metabolism	Low levels. No benefit over cotinine. Short term marker only
Minor tobacco alkaloids	Anatabine Anabasine	NDA	10–16 hr	Yes	Yes	NDA	Useful when individuals are using NRT; may be precursors to nitrosamines	Short-term marker only
Carbon monoxide	Exhaled CO	Yes	4–6 hr	Yes	No	Yes	Easy to measure in exhaled air	Other sources exist, including endogenous processes. Short-term marker only. Less reliably distinguishes levels of smoking among heavy smokers
	CO boost (pre- and post-cigarette levels)	Yes	NA	Yes	Yes	NDA	Measures exposure to single cigarette	Short term marker only. Levels vary over the day
	Carboxyhemoglobin	Yes	Hours	Yes	No	Yes	Measures cumulative, although short-term exposure to several cigarettes	Requires blood draw and special handling. Benefit above that for using exhaled CO not shown
Hydrogen cyanide	Thiocyanate	Yes	1–2 weeks	No	No	NDA	Long-term marker. Can be measured in urine, saliva, and blood. Saliva easy to obtain	Many dietary sources. Dose–response curve flattens at higher smoking levels so cannot distinguish among heavy smokers.
Tobacco-specific nitrosamines	Urinary metabolites	Yes	45 d	Yes	Yes	NDA	May reflect biologically effective dose	Technically difficult to measure
Polycyclic aromatic hydrocarbons	Parent compounds	NDA	NDA	Yes	No	Yes	Measured in organs where effect might occur	Technically difficult to obtain tissue and perform assay
	Urinary 3-hydroxypyrene and 1-hydroxypyrene	Yes	NDA	Yes	No	NDA	Assay simple to perform	Other exposures can be substantial

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Source: https://www.industrydocuments.ucsf.edu/docs/jnlx0001

Table 11-6 Biomarkers of Exposure[a,b]

Complex mixture assay	Urinary mutagenicity	Yes	Yes	No	No	NDA	May be related to in vivo mutagen exposure	Lack of specificity

NOTE: NA = not applicable; NDA = No data available.
[a]Selected examples; list is not all-inclusive.
[b]References are not provided in this table but can be found in the text of this and disease-related chapters.
[c]Any report related to a disease outcome associated with tobacco where the report is plausible but has not necessarily replicated.

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Source: https://www.industrydocuments.ucsf.edu/docs/jnlx0001

Table 11-7 Biomarkers Estimating the Biologically Effective Dose[a,b]

Category	Variables used in Literature	Dose–Response Data	Associated with Cessation or Half-life	Target Tissue Assay Available	Chemical Specificity	Specific to Tobacco	Related to a Disease Risk[c]	Strengths	Limitations
Carcinogen–DNA adducts	Nonidentified adducts/ ^{32}P-postlabeling	Yes	Yes	Yes	No	No	Yes	Facile assay; does not require knowledge of specific adducts; blood may be surrogate for lung tissue. Adducts found in all tissues, including heart and blood vessels	Cannot identify adducts so mechanistic studies are problematic
	PAH–DNA adducts	Yes	9–13 weeks (blood)	Yes	Yes	No	Yes	Can be measured in any tissue and assays are available that are sufficiently sensitive	Low sensitivity and technical difficulties make assay use limited in large-scale studies. Diet might be greater contributor than smoking
	4-Aminobiphenyl–DNA adducts	Yes	Yes	Yes	Yes	No	NDA	Can be measured in any tissue; has some specificity for smoking if no known occupational exposure	Low sensitivity makes assay use limited in large-scale studies
	NNK–DNA adducts	Yes	NDA	Yes	Yes	Yes	NDA	Can be measured in any tissue, although methodology has low sensitivity. Highly specific for smoking	Low sensitivity makes assay use limited in large-scale studies
	8-hydroxydeoxyguanosine	No	Yes	Yes	Yes	No	NDA	Can be measured in any tissue	Assay has large interlaboratory variation; it is easy to introduce oxidative damage into laboratory assay; low sensitivity makes assay use limited in large-scale studies
	5-(Hydroxymethyl)uracil	No	NDA	No	Yes	No	Not available	Sufficient sensitivity to use for ETS	Technically difficult
	N-Nitrosamine-related-DNA adducts	NDA	26 hr (blood; O6-methyldeoxyguanosine) and 60 hr (blood; 7-methyldeoxyguanosine)	Yes	Yes	No	NDA	Can be measured in any tissue	Low sensitivity makes assay use limited in large-scale studies. Diet a common source
Carcinogen–hemoglobin (Hgb) adducts	PAH–Hgb adducts	Yes	NDA	No	Yes	No	NDA	Large amount of adducts available in blood so method is facile	Surrogate assay not yet validated against target organ damage
	4-Aminobiphenyl–Hgb adducts	Yes	7–9 weeks	No	Yes	No	NDA	Large amount of adducts available in blood so method is facile	Surrogate assay not yet validated against target organ damage

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Source: https://www.industrydocuments.ucsf.edu/docs/jnlx0001

Table 11-7 Biomarkers Estimating the Biologically Effective Dose[a,b]

Category	Variables used in Literature	Dose–Response Data	Associated with Cessation or Half-life	Target Tissue Assay Available	Chemical Specificity	Specific to Tobacco	Related to a Disease Risk[c]	Strengths	Limitations
Carcinogen–protein adducts	PAH–albumen adducts	Yes	NDA	No	Yes	No	NDA	Large amount of adducts available in blood so method is facile	Surrogate assay not yet validated against target organ damage
Carcinogen–DNA adduct antibodies	Anti-BPDE serum antibodies	NDA	NDA	No	No	NDA	NDA	May provide long-term marker of exposure	Doubtful that a dose–response relationship can be established due to complexity of immune response in individuals
	Adducts	Yes	Yes		Yes	No	NDA	Measured in organs where effect might occur	Technically difficult to obtain tissue and perform assay
Carbon monoxide	Carboxyhemoglobin	Yes	Yes	No	Yes	No	Yes	Might also reflect a surrogate measure of biologically effective dose	Logistical problems in sample handling
Lipid peroxidation	F2-Isoprostanes	No	Yes	No	Yes	No	NDA	Corroborative end point for oxidative damage without artifactual introduction of oxidative damage	Technically difficult

NOTE: NA = not applicable; NDA = no data available; NNK = Nicotine-derived nitroketone ; BPDE = benzo(a)pyrene-diol-epoxide
[a]Selected examples; list is not all-inclusive.
[b]References are not provided in this table but can be found in the text of this and disease-related chapters.
[c]Any report related to a disease outcome associated with tobacco where the report is plausible but has not necessarily been replicated.

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Source: https://www.industrydocuments.ucsf.edu/docs/jnlx0001

Table 11-8 Biomarkers of Potential Harmful Effects[a,b]

Category	Variables Used in Literature	Dose–Response Data	Associated with Cessation or Half-life	Target Tissue Assay Available	Chemical Specificity	Specific to Tobacco	Related to a Disease Risk[c]	Strengths	Limitations
Enzymatic induction	Aryl hydrocarbon hydroxylase	No	>30 d	Yes	Yes	No	Yes	Indicates acquired changes in susceptibility; related to DNA–adduct levels	Technically difficult to assess in large epidemiological studies.
	CYP1A2	No	NDA	Yes	Yes	No	Yes	Indicates acquired changes in susceptibility; related to DNA–adduct levels	Technically difficult to assess in large epidemiological studies
	DNA repair enzymes	NDA	Yes	Yes	NA	No	NDA	Indicates acquired changes in susceptibility; provides analysis of what is likely to be critical part of carcinogenesis	Technically difficult
	Microarray assays for mRNA expression and proteomics	NDA	NDA	Yes	NA	No	NDA	Reflects integrated measure of multiple genotypes, provides complex data potentially usable for rapid identification of important risk factors	Difficult to perform; relationship to disease risk is technically difficult to prove; requires extensive laboratory validation; RNA and protein microarray assays are expensive; large-scale studies are needed; refined bioinformatics analysis required
Chromosomal alterations	Chromosomal aberrations	Yes	Yes	Yes	No	No	Yes	Can be done in blood as surrogate tissue. Similar lesions observed in cancer. Can be measured in persons without cancer	Very nonspecific; relationship to target organ is not established; significant lack of specificity and wide overlap between smokers and nonsmokers
	Micronuclei	Yes	Yes	Yes	No	No	NDA	Facile assay	Lack of specificity
	Sister chromatid exchanges	Yes	Yes	No	No	No	No	Easy to do in blood as surrogate tissue. Can be measured in persons without cancer	Very nonspecific; relationship to target organ is not established; predictivity for disease risk not established. Association with cancer in case-control studies may have case bias. Significant lack of specificity and wide overlap between smokers and nonsmokers
	Loss of heterozygosity	Yes	Yes	Yes	No	No	NDA	Similar lesions observed in cancer	Technically complex; relationship to cancer risk unknown
	Mutations in reporter genes (*HPRT, GPA*)	Yes	Yes	No	No	No	NDA	Facile assay in blood	Relationship to target tissue or blood unknown
	Mutational load in target genes (p53, K-ras)	NA	NDA	Yes	No	No	NDA	Target gene specificity	Very difficult to do in normal tissues
Mitochondrial mutations	Deletions, insertions	NDA	NDA	Yes	No	No	NDA	Provides corroborative marker	Relationship to disease not established

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Source: https://www.industrydocuments.ucsf.edu/docs/jnlx0001

Table 11-8 Biomarkers of Potential Harmful Effects[a,b]

Category	Variables Used in Literature	Dose–Response Data	Associated with Cessation or Half-life	Target Tissue Assay Available	Chemical Specificity	Specific to Tobacco	Related to a Disease Risk[c]	Strengths	Limitations
Epigenetic cancer effects	Whole genome methylation	NDA	NDA	Yes	No	No	No	Facile assay	Relationship to disease unknown
	Hypermethylation of promoter regions	NDA	NDA	Yes	No	No	No	Similar lesions observed in cancers	Technically difficult; relationship to risk unknown
Lipids	Blood lipids: HDL, LDL, oxidized LDL, triglycerides	Yes	NDA	Yes	Yes	No	Yes	May be directly related to disease risk	Levels among heavy smokers cannot be distinguished. Wide interindividual variation. Many individuals under medication therapy. Significant confounders exist
Cardiovascular response	Heart rate, blood pressure	No	Yes	Yes	NA	No	Yes	Easy to measure; intraindividual differences may be important for the individual	Both interindividual and intraindividual differences are significant. Substantial confounders exist, and many persons are on medications
Thrombosis	Bleeding time	No	NDA	Yes	No	No	No	Minimally invasive	Very nonspecific
	Fibrinogen	NDA	NDA	Yes	Yes	No	NDA	Pathogenically related to disease	Does not distinguish levels of smoking. Nicotine might separately affect these parameters so limited use in persons using NRT
	Prothrombin time, partial thromboplastin time, plasminogen activator inhibitor, C-reactive protein	Yes	NDA	Yes	Yes	No	NDA	Leave fingerprint at site of their formation	
	Urinary thromboxane and prostacyclins	Yes	No	No	Yes	No		May be markers of platelet aggregation; reflect chronic exposure	Kidney disease can affect urinary excretion. Technically difficult. Wide overlap of values due to individual differences in response
	Platelet activation and survival	Yes	NDA	Yes	No	No	No	Platelet activation in vivo might be pathophysiologically related to cardiac artery thrombosis	Technically difficult to use for large numbers of subjects. Significant number of confounding variables. Smoking increases platelet counts
Blood cell parameters	White blood cell counts (i.e., lymphocytes, neutrophils, total counts)	Yes	Yes	Yes	Yes	No	Yes	Can be a surrogate marker for several processes including atherosclerosis and thrombosis	Relationship to disease uncertain, although alterations in levels are linked epidemiologically to disease. Wide interindividual and intraindividual variation and large number of confounders
	Hematocrit, hemoglobin, red blood cell mass	Yes	Yes	Yes	No	No	No	Can reflect both cardiac and respiratory disease risk	Insensitive; wide interindividual differences

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Source: https://www.industrydocuments.ucsf.edu/docs/jnlx0001

Table 11-8 Biomarkers of Potential Harmful Effects[a,b]

Category	Variables Used in Literature	Dose–Response Data	Associated with Cessation or Half-life	Target Tissue Assay Available	Chemical Specificity	Specific to Tobacco	Related to a Disease Risk[c]	Strengths	Limitations
Bronchioalveolar lavage response	Inflammatory cells, protein, cytokines	Yes	Yes	Yes	No	No	NDA	Provides different types of data with single procedure	Bronchoscopy is too invasive for large epidemiological studies
	Neutrophil elastase α1-antiprotease complex	Yes	Yes	Yes	No	No	NDA	Provides different types of data with single procedure	Bronchoscopy is too invasive for large epidemiological studies
	α1-antitrypsin	No	No	Yes	Yes	Yes	NDA	May be specific to tobacco smoke	Requires invasive test; short half-life
Inflammatory mediators of response	Leukotrienes	Yes	NDA	No	Yes	No	NDA	May be measured in urine, bronchioalveolar lavage, and serum	Substantial number of confounders
Pulmonary function tests	FEV1, FVC	Yes	Yes	Yes	No	No	Yes	Widely available	Low sensitivity for mild disease. Decrease in function with aging. Large interindividual variation
Periodontal disease	Periodontal height	Yes	Yes	Yes	No	No	Yes		
	Gum bleeding	Yes	Yes	Yes	No	No	Yes		
Osteoporosis	Fractures	Yes	NDA	NA	No	No	Yes	Easily measured	Numerous confounders
	Bone density	NDA	NDA	Yes	No	No	NA		
Skin	Premature wrinkling	Yes	NDA	NA	No	No	NA		
Fetal and neonatal effects	Birth weight	Yes	Yes	Yes	No	No	Yes	Data collection is easy	Nonspecific; numerous confounders
Weight	Weight loss and gain	Yes	Yes	Yes	No	No	Yes	Both a biomarker for metabolism and an important outcome for some people.	Some people perceive weight loss as a benefit of smoking, despite significant adverse effects associated with smoking

NOTE: NA = not applicable; NDA = no data available; FVC = Forced vital capacity; FEV_1 = Forced expiratory volume in 1 sec; HDL= high-density lipoprotein; LDL = low-density lipoprotein; HPRT = hypoxanthine phosphoribosyltransferase

[a]Selected examples; list is not all-inclusive.

[b]References are not provided in this table but can be found in the text of this and disease-related chapters.

[c]Any report related to a disease outcome associated with tobacco where the report is plausible but has not necessarily been replicated.

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Source: https://www.industrydocuments.ucsf.edu/docs/jnlx0001

Table 11-9 Assays for Assessing Effect Modification by Heritable Traits

Assay Type	Example Used in Literature	Strengths	Limitations
Gene-based assays	Genetic polymorphisms for carcinogen metabolism and induction or DNA repair, smoking behavior	Inexpensive, simple to perform, specific gene effect when exists, high throughput available	Functional relationship of genotype to phenotype difficult to prove; disease risk for low-penetrant genes difficult to prove
Phenotypic assays	Mutagen sensitivity for DNA repair; host-reactivation assay for DNA repair; CYP450 metabolism and induction studies; RNA expression of specific genes; microarray RNA expression; proteomics	Reflects integrated measure of multiple genotypes; provides complex data potentially usable for rapid identification of important risk factors	Difficult to perform; relationship to disease risk technically difficult to prove; requires extensive laboratory validation; RNA and protein microarray assays are expensive; large-scale studies are needed; bioinformatics not available

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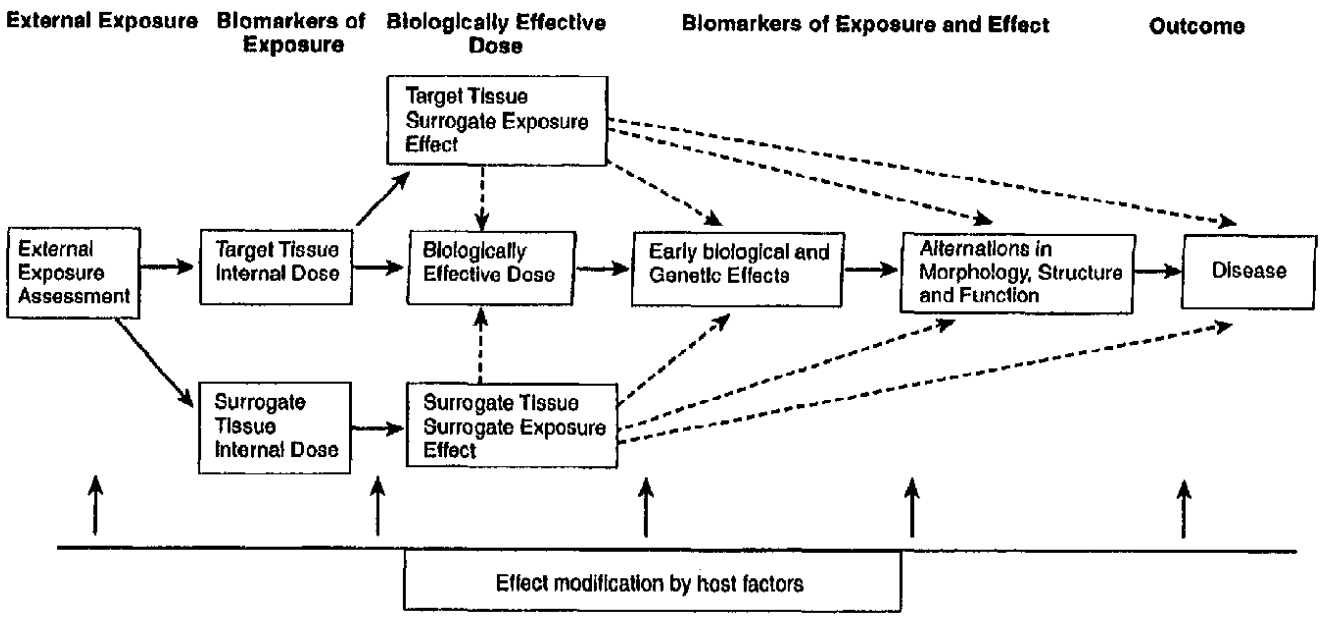
Source: https://www.industrydocuments.ucsf.edu/docs/jnlx0001

Figure 11-1 Assessing potential harm reduction products



NOTE: Dashed lines indicate hypothetical indirect relationship; Solid lines indicate mechanistic direct relationship.

SOURCE: Modified with permission from Environmental Health Perspectives. Committee on Biological Markers of the National Research Council. Biological Markers in Environmental Health Research. 74:3-9, 1987.

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Source: https://www.industrydocuments.ucsf.edu/docs/jnlx0001